Viral Infection Increases the Risk of Idiopathic Pulmonary Fibrosis: A Meta-analysis

Gaohong Sheng, M.D.[1#], Peng Chen, M.D., Ph.D.[2, 3#], Yanqiu Wei, M.D.[4], Huihui Yue, M.D.[4], Jiaojiao Chu, M.D.[4], Jianping Zhao, M.D., Ph.D.[4], Yihua Wang, M.D., Ph.D.[5,6], Wanguang Zhang, M.D., Ph.D.[7*], Hui-Lan Zhang, M.D., Ph.D.[4*]

[1]Department of Orthopedics, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Jiefang Avenue 1095, Wuhan 430030, China; [2]Division of Cardiology, Departments of Internal Medicine and Genetic Diagnosis Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology; [3]Hubei Key Laboratory of Genetics and Molecular Mechanism of Cardiological Disorders, Wuhan 430030, China; [4]Department of Respiratory and Critical Care Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095, Jie Fang Road, Han Kou District, Wu Han, HuBei Province 430030, China; [5]Biological Sciences, Faculty of Environmental and Life Sciences, University of Southampton, Southampton, SO17 1BJ, UK; [6]Institute for Life Sciences, University of Southampton, Southampton, SO17 1BJ, UK; [7]Hepatic Surgery Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, HuBei Province 430030, China

Brief Title: Viral Infection Increases the Risk of IPF

[#]Gaohong Sheng and Peng Chen contributed equally to this work.

*Corresponding author:

Hui-Lan Zhang, Department of Respiratory and Critical Care Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095, Jie Fang Road, Han Kou District, Wu Han City, Hu Bei Province 430030, China (Tel: 86-027-83663355, Email: huilanz_76@163.com)

Wanguang Zhang, Hepatic Surgery Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095, Jie Fang Road, Han Kou District, Wuhan, HuBei Province 430030, China (Tel: 86-027-83663603, Email: wanguangz@tjh.tjmu.edu.com)

Abstract

Background

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, fibrotic lung disease with a poor prognosis. Although many factors have been identified to possibly trigger or aggravate IPF, such as viral infection, the exact etiology of IPF remains unclear. Until now, there has been no systematic review to quantitatively assess the role of viral infection in IPF.

Objective

This meta-analysis aims to present a collective view on the relationship between viral infection and IPF.

Methods

We searched studies reporting the effect of viral infection on IPF from Pubmed, Embase, Cochrane Library, Web of Science, and Wiley Online Library databases. The value of OR with 95% CI was calculated to assess the risk of virus in IPF. We also estimated statistical heterogeneity by I^2 and Cochran Q test, publication bias by funnel plot, Begg's test, Egger's test and trim and fill method. Regression, sensitivity and subgroup analyses were performed to assess the effects of confounding factors, such as gender and age.

Results

We analyzed 20 case-control studies with 1287 participants from 10 countries. The pooled OR of all viruses indicated that viral infection could significantly increase the risk of IPF (OR: 3.48, 95% CI: 1.61-7.52, p=0.001), but not that of

exacerbation of IPF (OR: 0.99, 95% CI: 0.46-2.12, p=0.988). In addition, all analyzed viruses including Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 7 (HHV7) and human herpesvirus 8 (HHV8) were associated with a significant elevation in the risk of IPF, except human herpesvirus 6 (HHV6).

Conclusions

The presence of persistent or chronic, but not acute, viral infections including EBV, CMV, HHV7 and HHV8, significantly increases the risk of developing IPF, but not exacerbation of IPF. These findings imply that viral infection could be a potential risk factor for IPF.

Key words: Idiopathic pulmonary fibrosis, viral infection, virus, meta-analysis.

Abbreviations

ATS, American Thoracic Society

CFA, cryptogenic fibrosing alveolitis

CMV, cytomegalovirus

CI, confidence intervals

EBV, Epstein-Barr virus

EMT, Epithelial Mesenchymal Transition

ERS, European Respiratory Society

HBoV, Human Boca virus subtype 1

HCV, hepatitis C virus

HHV6, human herpesvirus 6

HHV7, human herpesvirus 7

HHV8, human herpesvirus 8

ICTV, International Committee on Taxonomy of Viruses

IFN-α, interferon-α

IFN-γR-/-, IFN-γR–deficient mice

ILD, interstitial lung disease

IPF, idiopathic pulmonary fibrosis

KSHV, Kaposi's sarcoma-associated herpes virus

LMP1, latent membrane protein 1

MHV68, murine γ-herpesvirus 68

NOS, Newcastle-Ottawa Scale

OR, odds ratio

PCR, polymerase chain reaction

PRISMA, preferred reporting items for systematic reviews and meta-analysis

RSV, respiratory syncytial virus

TGFβ1, transforming growth factor-β1

TTV, torque teno virus

Introduction

Interstitial lung diseases (ILD) is a varying group of disorders with pulmonary parenchyma involvement; idiopathic pulmonary fibrosis (IPF) is one of the major idiopathic ILD. IPF, with an incidence of 2.8-9.3 per 100000 per year, is a chronic, progressive and fibrotic lung disease characterized by fibroblast proliferation, extracellular matrix accumulation and destruction of pulmonary architecture.[1,2] It is prone to occur in men and those who are older than 50-years.[3-5] In severe cases, it develops into restrictive pulmonary ventilatory dysfunction, impaired gas exchange, and even respiratory failure.[6-8] The prognosis of IPF is poor, with the median survival after diagnosis generally estimated at 2-5 years, although it may be prolonged to 6.87-7.91 years under specific antifibrotic therapy.[9-13] Although many studies have focused on IPF, the etiology of IPF still remains unclear.

In addition to genetic factors,[14-17] a variety of environmental exposures have been identified to be closely related to the initiation and progression of IPF, including cigarette smoking, metal and wood dusts exposure, silica and agricultural surroundings, and microbial infections.[18-20] Among these factors, the relationship between virus and IPF is widely investigated, and accumulating evidence implies that viral infections may play an important role in the initiation and exacerbation of IPF.[21,22] However, the exact pathogenetic relationship between viral infection and IPF remains the subject of ongoing investigation.[1,4,23-25]

The purpose of this meta-analysis is to estimate the association between

viral infections and the development or exacerbation of IPF.

Methods

Search strategy and selection criteria

The search flow diagram of this meta-analysis is presented in **Figure 1**. This study was performed according to the standards set forth by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement.[26] In this meta-analysis, we searched Pubmed, Embase, Cochrane Library, Web of Science, Wiley Online Library databases, conference proceedings and other unpublished studies with terms “idiopathic pulmonary fibrosis”, “IPF”, “cryptogenic fibrosing alveolitis”, “CFA”, and “virus”. All included studies were published in English up to December 31, 2018. In addition, the related articles in references were manually searched.

Inclusion criteria

We included controlled studies in English on patients diagnosed with IPF and acute exacerbation of IPF according to the American Thoracic Society (ATS) and European Respiratory Society (ERS) statements[3,4,27,28] and the reports of Collard et al, respectively.[29,30] Viral infection had to be detected by laboratory examinations, with the methodlogy of virus detection described in detailed. We only included studies with at least 3 participants in the control group.

Exclusion criteria

Uncontrolled studies or studies lacking data regarding the control group were

excluded. Studies were not eligible if all the participants were detected as virus positive/negative in both case and control groups, because the odds ratio (OR) could not be calculated.[31,32] Studies that reported mean antibody titers instead of negative or positive results of virus detection were also eliminated. In addition, studies which recruited participants after lung or bone marrow transplantation were excluded.

After full-text assessments of 48 studies, a total of 28 were excluded. Six studies were excluded because they divided participants into case and control groups according to the presence or absence of virus infection instead of IPF diagnosis. Eight studies were excluded due to absence of control group. Four studies were excluded because virus detection outcomes were positive or negative in all participants. Another study were excluded because it only provided the levels of mean antibody titer. We further removed 9 studies where IPF diagnostic criteria did not refer to ATS/ERS statements. Finally, 20 eligible studies were included and analyzed in this meta-analysis.

Data extraction

Literature search and data extraction were independently performed by two researchers (Gaohong Sheng and Peng Chen). Disagreement between the two researchers were settled by arbitration of the principal investigator (Hui-Lan Zhang). The characteristics of studies and participants, and data of virus detection were collected and analyzed. Furthermore, the data of acute exacerbation of IPF were collected and analyzed as well.

Quality Assessment

The Newcastle-Ottawa Scale (NOS) was used to assess the quality of all included studies.[33] Each study was assessed by two researchers independently. The studies with a NOS score of 5 stars or more are considered as high quality and qualified to be included and analyzed in this meta-analysis. To estimate whether control groups were adequately selected, those ones that utilized healthy volunteers as control were rendered a star by NOS. For the item of comparability, studies which satisfied the most important factor would be given a star. One star would be appraised if the age gap between the case and control groups is less than or equal to 10 years. Since the second important factor is the sex ratio, one star would be given to a study where the value of sex ratio is within the range of 1/2 - 2 by control group dividing case group.

Statistical Analysis

We performed this meta-analysis using OR with 95% confidence intervals (CI) as the effect measure. In addition, in the studies with zero events in only one group, 0.5, the continuity correction was added to each cell of the table. Mantel–Haenszel method was used to estimate the pooled OR in order to reduce the bias.[31,32] Methods of sample collection and viral detection varied among studies. We preferentially extracted results that used polymerase chain reaction (PCR) with lung tissue samples. The IgM level was used only when PCR data were not available.

We evaluated publication bias using funnel plot, Egger's regression asymmetry test and Begg's adjusted rank correlation test. When obvious publication bias was found, we used trim and fill method to adjust and confirm our results.[34,35] The heterogeneity of study was assessed using Cochran'Q statistic, and its magnitude was estimated using I^2 statistic. Random effect model was used when significant heterogeneity was observed ($p < 0.1$ or $I^2 > 50\%$), otherwise we used fixed effect model. Sensitivity analyses was performed to examine stability of pooled outcome. In addition, Meta regression and subgroup analyses were performed according to the mean age of patients and the proportion of man.

All comparisons were two-tailed, and $p < 0.05$ was recognized as statistical significance. STATA software 12.0 was used to perform statistical analyses.

Results

Characteristics of studies and patients

This meta-analysis reviewed and analyzed 20 case-control studies published from 1984 to 2018, covering 1287 participants from 10 countries (**Table 1**) (634 IPF cases and 653 controls). The mean age of participants was 59.75 years old. The mean proportion of males was 69% (**Table 2**). More detailed characteristics of the studies and patients are shown in **Table 1** and **Table 2**. All included studies were distinguished as high-quality studies by NOS quality assessments (**Supplementary Table 1**). According to the virus classification of International Committee on Taxonomy of Viruses (ICTV), a total of 19 virus species were detected using various laboratory examinations. Among them, five species of virus had more than two sets of data, including Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 6 (HHV6), human herpesvirus 7 (HHV7), and human herpesvirus 8 (HHV8) (**Supplementary Table 2**).

Relationship between virus infection and IPF

All viruses

For all species of virus, we analyzed the data of viral infection in 634 patients from 20 studies. A significant elevation associated with viral infection was found in the risk of IPF (OR: 3.48, 95% CI: 1.61-7.52, p=0.001) (**Figure 2a**). The heterogeneity was I^2: 76.0% (p<0.001). Publication bias was found in the funnel plot (**Supplementary Figure 1a**). The p value of Begg's test was 0.004

(**Supplementary Figure 1b**), while that of Egger's test p=0.001 (**Supplementary Figure 1c**). Considering the influence of publication bias, we conducted trim and fill method (**Supplementary Figure 1d**) and the result of OR (1.64, 95% CI: 0.73-3.69, p=0.231) failed to confirm the stability of this effect.[36] Almost all studies published before 2000 were removed due to inability to apply ATS/ERS guidelines, which likely led to this publication bias. Sensitivity analysis examined the robustness by evaluating the impacts of small sample studies (**Supplementary Figure 1e**) and individual studies (**Supplementary Figure 1f**) on pooled effects.

EBV

EBV is a typical species of the Gammaherpesvirinae family. A total of 10 studies consisting of 252 patients reported data on EBV infection, and EBV infection was associated with a significant elevation in the risk of IPF (OR: 9.83, 95% CI: 4.22-22.91, p<0.001) (**Figure 2b**). The degree of the heterogeneity was I^2: 41.5% (p=0.081). Publication bias was not negligible from the funnel plot (**Supplementary Figure 2a**), and also detected by Begg's test (p=0.032) (**Supplementary Figure 2b**) as well as Egger's test (p=0.030) (**Supplementary Figure 2c**). Considering the influence of publication bias, we conducted trim and fill method (**Supplementary Figure 2d**) and the result of OR (6.16, 95% CI: 2.74-13.86, p<0.001) further confirmed the stability of our results. No obvious influences were found from small sample studies (**Supplementary Figure 2e**) or individual studies through sensitivity analyses

(**Supplementary Figure 2f**).

CMV

CMV is a common virus which belongs to the Betaherpesvirinae family, and a total of 209 patients from 9 studies were analyzed. We evaluated the pooled effect and found infection of CMV contributing significantly to IPF risk (OR: 2.45, 95%CI: 1.29-4.66, p=0.006) (**Figure 2c**). The degree of the heterogeneity was I^2: 24.1% (p=0.229). No obvious publication bias was observed from funnel plot (**Supplementary Figure 3a**), quantitative Begg's test (p=0.754) (**Supplementary Figure 3b**) or Egger's test (p=0.355) (**Supplementary Figure 3c**). Neither small sample studies (**Supplementary Figure 3d**) nor individual studies (**Supplementary Figure 3e**) had significant impacts on the pooled OR by sensitivity analysis.

HHV6

Three studies involving 80 patients provided data on HHV6, which also belongs to the Betaherpesvirinae family. The result of pooled OR showed no statistically significant elevation in risk of IPF (OR: 3.16, 95%CI: 0.41-24.50, p=0.270) (**Figure 2d**). The degree of the heterogeneity was I^2: 71.4% (p=0.030). The funnel plot (**Supplementary Figure 4a**) showed no evident publication bias by qualitative evaluation, and the quantitative assessments via Begg's test (p=1.000) (**Supplementary Figure 4b**) and Egger's test (p=0.242) (**Supplementary Figure 4c**). Sensitivity analysis did not show prominent influences on pooled effect from small sample studies (**Supplementary**

Figure 4d) or individual studies (**Supplementary Figure 4e**).

HHV7

Another member of Betaherpesvirinae family, HHV7 was investigated in 4 studies with 95 patients. Pooled effect showed patients with HHV7 infection had higher risk of IPF (OR: 2.48, 95%CI: 1.14-5.40, p=0.022) (**Figure 2e**). The degree of the heterogeneity was I^2: 0.0% (p=0.625). No publication bias was found by funnel plot (**Supplementary Figure 5a**), Begg's test (p=0.308) (**Supplementary Figure 5b**) and Egger's test (p=0.702) (**Supplementary Figure 5c**). Sensitivity analysis showed no significant influences on overall effect, including small sample studies (**Supplementary Figure 5d**) and individual studies (**Supplementary Figure 5e**).

HHV8

HHV8 is a species which belongs to the Gammaherpesvirinae family, also known as Kaposi's sarcoma-associated herpes virus (KSHV).[37] We found 2 studies of HHV8 and 1 study of KSHV to obtain the overall effect of OR, and the pooled effect showed that HHV8 infection increased the risk of IPF significantly (OR: 8.88, 95%CI: 2.70-29.14, p<0.001) (**Figure 2f**). The degree of heterogeneity was I^2: 0.0% (p=0.662). No publication bias was found by the funnel plot (**Supplementary Figure 6a**), Begg's test (p=1.000) (**Supplementary Figure 6b**) or Egger's test (p=0.508) (**Supplementary Figure 6c**). Sensitivity analysis for overall effect showed no significant influences from small sample studies (**Supplementary Figure 6d**) or

individual studies (**Supplementary Figure 6e**).

Relationship between multiple viral infections and IPF

Six studies containing 174 patients provided data on multiple viral infections. The result of pooled OR did not show that multiple viral infections increased risk of IPF significantly (OR: 3.37, 95%CI: 0.69-16.44, p=0.133) (**Figure 3a**). The degree of the heterogeneity was I^2: 60.4% (p=0.027). The funnel plot (**Supplementary Figure 7a**) showed no apparent publication bias by qualitative evaluation and the quantitative assessments by Begg's test (p=0.707) (**Supplementary Figure 7b**) and Egger's test (p=0.284) (**Supplementary Figure 7c**). Sensitivity analysis showed that influences on pooled effect were not remarkable from small sample studies (**Supplementary Figure 7d**) or individuals studies (**Supplementary Figure 7e**).

Relationship between viral infection and acute exacerbation of IPF

A total of 3 studies provided data on acute exacerbation of IPF patients. However, viral infection was not found to associate with acute exacerbation of IPF (OR: 0.99, 95%CI: 0.46-2.12, p=0.988) (**Figure 3b**). The degree of heterogeneity was I^2: 0.0% (p=0.759). No publication bias was found in the funnel plot (**Supplementary Figure 8a**), Begg's test (p=0.296) (**Supplementary Figure 8b**) and Egger's test (p=0.209) (**Supplementary Figure 8c**). Sensitivity analysis showed no obvious influences caused by small sample studies (**Supplementary Figure 8d**) or individual studies (**Supplementary Figure 8e**).

Meta regression

Age and gender were used as covariates for Meta regression analyses and the results suggested that the viral infection related to IPF in an age and gender independent manner ($p>0.05$ for all) (**Supplementary Table 3**).

Subgroup analyses

We also performed subgroup analyses based on the mean age of patients and the proportion of males for all viruses, EBV CMV and viruses of multiple infections, with no significant difference between subgroups was found (**Supplementary Table 4**).

Discussion

This meta-analysis including 20 case-control studies with 634 IPF cases and 653 controls, covering 19 virus species, aimed to evaluate the relationship between viral infection and IPF. We found that viral infection was associated with higher risk of IPF but not of acute exacerbation of IPF. Further, we performed subgroup analyses to evaluate the relationship between IPF and each kind of virus, including EBV, CMV, HHV6, HHV7 and HHV8. The results showed that EBV, CMV, HHV7 and HHV8, but not HHV6, were associated with a significant increase in the risk of IPF.

So far, the exact etiology and pathogenesis of IPF still remains unclear. Accumulating studies have focused on the role of viral infection in IPF patients, but the interpretation of them was hindered by their relatively small sample size and differing populations studied.[21,22,38,39]

Historically, “IPF” comprises a heterogeneous group of different histological and clinical entities arising in an idiopathic setting. Before ATS/ERS refined IPF as those patients with usual interstitial pneumonia (UIP) pattern on lung biopsy or high-resolution computed tomography (HRCT), the diagnosis of IPF was imprecise and variable,[3,4,6,27] which is a major pitfall of this meta-analysis. Therefore, we compared the outcomes between all studies related to “IPF” and those referring to ATS/ERS statements in **Supplementary Table 5**. There were no dramatic changes in any results for all viruses, EBV, CMV, HHV6, HHV7 and HHV8. In addition, the stability of the result of all studies involving “IPF” for all viruses was confirmed by the trim and fill method, while it was not

confirmed in the result of studies using the ATS/ERS criteria. This implies that the publication bias of studies using the ATS/ERS criteria might influence the overall results. The “IPF” patients in those excluded studies might accord with ATS/ERS statements, since they were all published earlier than publication of these statements. The characteristics of studies involving “IPF” but not definitely referring to ATS/ERS statements are shown in **Supplementary Table 6**.

We further analyzed and calculated OR for patients with multiple chronic viral infections but did not find a statistically significant increase in the risk of IPF. The species and amount of viruses detected in different studies could result in bias, as humans are likely to harbor multiple persistent viruses without obvious symptoms throughout their lifetimes.[40] Thus, further study are needed to detect more viruses in IPF patients in order to assess the effect of multiple viral infections on IPF.

The result of subgroup analysis of age suggested the possibility that younger participants with viral infection might exhibit higher risk of IPF, although the interaction term for age (p=0.186) was not significant. It seemed that IPF of the young might be influenced by factors that differ from the elderly. The changes in the lung with age could facilitate the development of lung disease, including pulmonary fibrosis and lung infections.[41] Moreover, Naik et al suggested that aged lung may be particularly susceptible to viral-induced fibrosis.[38] However, the role of age is still poorly understood in viral infection

related IPF, and more works are needed to clarify in the future.

Previous studies have reported that murine models of viral infection could foster lung fibrogenesis.[42,43] However, the murine models predominantly focus on the role of acute inflammation driven by viral infection. Virgin et al have compared the differences between chronic and acute viral infections.[40] Thus, it should be noted that the majority of the viruses analyzed in our study are chronic/persistent viruses that are likely to have been acquired prior to the development of IPF. In view of this, we must be cautious regarding the differences in the pathogenesis of pulmonary fibrosis between humans and murine models. More appropriate animal models are requisite to further reveal the pathogenetic relationship between viral infection and IPF.

Malizia et al suggested that EBV-infected epithelial cell may play a role in IPF via transforming growth factor-β1 (TGFβ1) and CUX1/Wnt signaling pathway, which were involved in Epithelial Mesenchymal Transition (EMT), the mechanism of fibrosis production in tissue.[44,45] Human Boca virus subtype 1 (HBoV) might trigger development of pulmonary fibrosis by regulating the expression of cytokines.[23] It was also reported that latent membrane protein 1 (LMP1) expressed by EBV could induce EMT in synergy with TGFβ1.[46] Meanwhile, our results showed that infection of EBV was associated with a significant elevation in the risk of IPF, in agreement with previous studies.

Studies have reported that antiviral drugs against EBV and herpesvirus could stabilize the condition of patients with IPF.[16,47] Additionally, interferon-α

(IFN-α) treatment revealed substantial anti-fibrotic effects in a mouse model.[48] These studies hint that specific therapy towards culpable viral infections might be of therapeutic use.

All studies included in this meta-analysis are retrospective case-control studies with limitation of explaining causal relationships. The sample size in some subgroups is small. In addition, the viral infection rates are variable due to the sites of biological samples and multiple virus detection techniques. Different methods lead to alterations in sensitivity and specificity. Given these challenges, larger-scale samples and higher quality studies are needed in the future to draw conclusions on the exact causal relationship between the virus and IPF and/or virus and acute exacerbation of IPF.

In conclusion, the presence of viral infections associated with a significant increase in the risk of IPF, specifically for EBV, CMV, HHV7 and HHV8 infections. Our study supports the hypothesis that virus infection could play a key role in pathogenesis of IPF.

Acknowledgements: The authors are grateful for all the participants in this study.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Hui-Lan Zhang was the guarantor of the entire manuscript for designing and supervising the entire study. Gaohong Sheng and Peng Chen independently completed the literature retrieval and data extraction. The manuscript was drafted by Gaohong Sheng and checked and approved by Wanguang Zhang. Yanqiu Wei addressed grammatical/wording issues of the manuscript to smooth it out. Huihui Yue contributed the tables and figures. Jiaojiao Chu was in charge of statistical methods in this study. Jianping Zhao and Yihua Wang provided guidance for writing the part of Discussion.

Funding

This study was supported by NSFC projects (No. 81874149 and No. 81974456), Clinical Research Physician Program - of Tongji Medical College, HUST (No. 5001540075), and an Academy of Medical Sciences/the Wellcome Trust Springboard Award [SBF002/1038].

Reference

1. Hutchinson J, Fogarty A, Hubbard R, McKeever T. Global incidence and mortality of idiopathic pulmonary fibrosis: a systematic review. *Eur Respir J.* 2015;46(3):795-806.
2. Samet JM, Coultas D, Raghu G. Idiopathic pulmonary fibrosis: tracking the true occurrence is challenging. *Eur Respir J.* 2015;46(3):604-606.
3. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med.* 2011;183(6):788-824.
4. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). *Am J Respir Crit Care Med.* 2000;161(2 Pt 1):646-664.
5. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2006;174(7):810-816.
6. Fellrath JM, du Bois RM. Idiopathic pulmonary fibrosis/cryptogenic fibrosing alveolitis. *Clin Exp Med.* 2003;3(2):65-83.
7. Richeldi L, Collard HR, Jones MG. Idiopathic pulmonary fibrosis. *The Lancet.* 2017;389(10082):1941-1952.
8. Lederer DJ, Martinez FJ. Idiopathic Pulmonary Fibrosis. *N Engl J Med.* 2018;378(19):1811-1823.
9. Ley B, Collard HR, King TE, Jr. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2011;183(4):431-440.
10. Noble PW, Albera C, Bradford WZ, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. *The Lancet.* 2011;377(9779):1760-1769.
11. Jo HE, Glaspole I, Grainge C, et al. Baseline characteristics of idiopathic pulmonary fibrosis: analysis from the Australian Idiopathic Pulmonary Fibrosis Registry. *Eur Respir J.* 2017;49(2).
12. Margaritopoulos GA, Trachalaki A, Wells AU, et al. Pirfenidone improves survival in IPF: results from a real-life study. *BMC Pulm Med.* 2018;18(1):177.
13. Fisher M, Nathan SD, Hill C, et al. Predicting Life Expectancy for Pirfenidone in Idiopathic Pulmonary Fibrosis. *J Manag Care Spec Pharm.* 2017;23(3-b Suppl):S17-S24.
14. Armanios MY, Chen JJ, Cogan JD, et al. Telomerase mutations in families with idiopathic pulmonary fibrosis. *N Engl J Med.* 2007;356(13):1317-1326.
15. Nogee LM, Dunbar AE, 3rd, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med.* 2001;344(8):573-579.
16. Stuart BD, Choi J, Zaidi S, et al. Exome sequencing links mutations in PARN and RTEL1 with familial pulmonary fibrosis and telomere shortening. *Nat Genet.* 2015;47(5):512-517.
17. Kropski JA, Lawson WE, Young LR, Blackwell TS. Genetic studies provide clues on the pathogenesis of idiopathic pulmonary fibrosis. *Dis Model Mech.* 2013;6(1):9-17.
18. Ryu JH, Colby TV, Hartman TE, Vassallo R. Smoking-related interstitial lung diseases: a concise review. *Eur Respir J.* 2001;17(1):122-132.
19. Hubbard R, Lewis S, Richards K, Johnston I, Britton J. Occupational exposure to metal or wood dust and aetiology of cryptogenic fibrosing alveolitis. *Lancet.* 1996;347(8997):284-289.
20. Hubbard R. Occupational dust exposure and the aetiology of cryptogenic fibrosing alveolitis.

Eur Respir J Suppl. 2001;32:119s-121s.

21. Gan JJE, Woodcock AA, Stewart JP. Viruses and idiopathic pulmonary fibrosis. *European Respiratory Journal.* 1997;10(7):1433-1437.
22. Moore BB, Moore TA. Viruses in Idiopathic Pulmonary Fibrosis. Etiology and Exacerbation. *Annals of the American Thoracic Society.* 2015;12 Suppl 2:S186-192.
23. Khalfaoui S, Eichhorn V, Karagiannidis C, et al. Lung Infection by Human Bocavirus Induces the Release of Profibrotic Mediator Cytokines In Vivo and In Vitro. *PLoS One.* 2016;11(1):e0147010.
24. Kubo K, Yamaguchi S, Fujimoto K, et al. Bronchoalveolar lavage fluid findings in patients with chronic hepatitis C virus infection. *Thorax.* 1996;51(3):312-314.
25. Arase Y, Suzuki F, Suzuki Y, et al. Hepatitis C virus enhances incidence of idiopathic pulmonary fibrosis. *World Journal of Gastroenterology.* 2008;14(38).
26. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med.* 2009;6(7):e1000100.
27. American Thoracic S, European Respiratory S. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am J Respir Crit Care Med.* 2002;165(2):277-304.
28. Raghu G, Remy-Jardin M, Myers JL, et al. Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med.* 2018;198(5):e44-e68.
29. Collard HR, Moore BB, Flaherty KR, et al. Acute exacerbations of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2007;176(7):636-643.
30. Collard HR, Ryerson CJ, Corte TJ, et al. Acute Exacerbation of Idiopathic Pulmonary Fibrosis. An International Working Group Report. *Am J Respir Crit Care Med.* 2016;194(3):265-275.
31. Friedrich JO, Adhikari NK, Beyene J. Inclusion of zero total event trials in meta-analyses maintains analytic consistency and incorporates all available data. *BMC Med Res Methodol.* 2007;7:5.
32. Sweeting MJ, Sutton AJ, Lambert PC. What to add to nothing? Use and avoidance of continuity corrections in meta-analysis of sparse data. *Stat Med.* 2004;23(9):1351-1375.
33. Viale L, Allotey J, Cheong-See F, et al. Epilepsy in pregnancy and reproductive outcomes: a systematic review and meta-analysis. *Lancet.* 2015;386(10006):1845-1852.
34. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Performance of the trim and fill method in the presence of publication bias and between-study heterogeneity. *Stat Med.* 2007;26(25):4544-4562.
35. Mavridis D, Salanti G. How to assess publication bias: funnel plot, trim-and-fill method and selection models. *Evidence Based Mental Health.* 2014;17(1):30-30.
36. Hayashino Y, Noguchi Y, Fukui T. Systematic evaluation and comparison of statistical tests for publication bias. *J Epidemiol.* 2005;15(6):235-243.
37. Russo JJ, Bohenzky RA, Chien MC, et al. Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). *Proc Natl Acad Sci U S A.* 1996;93(25):14862-14867.

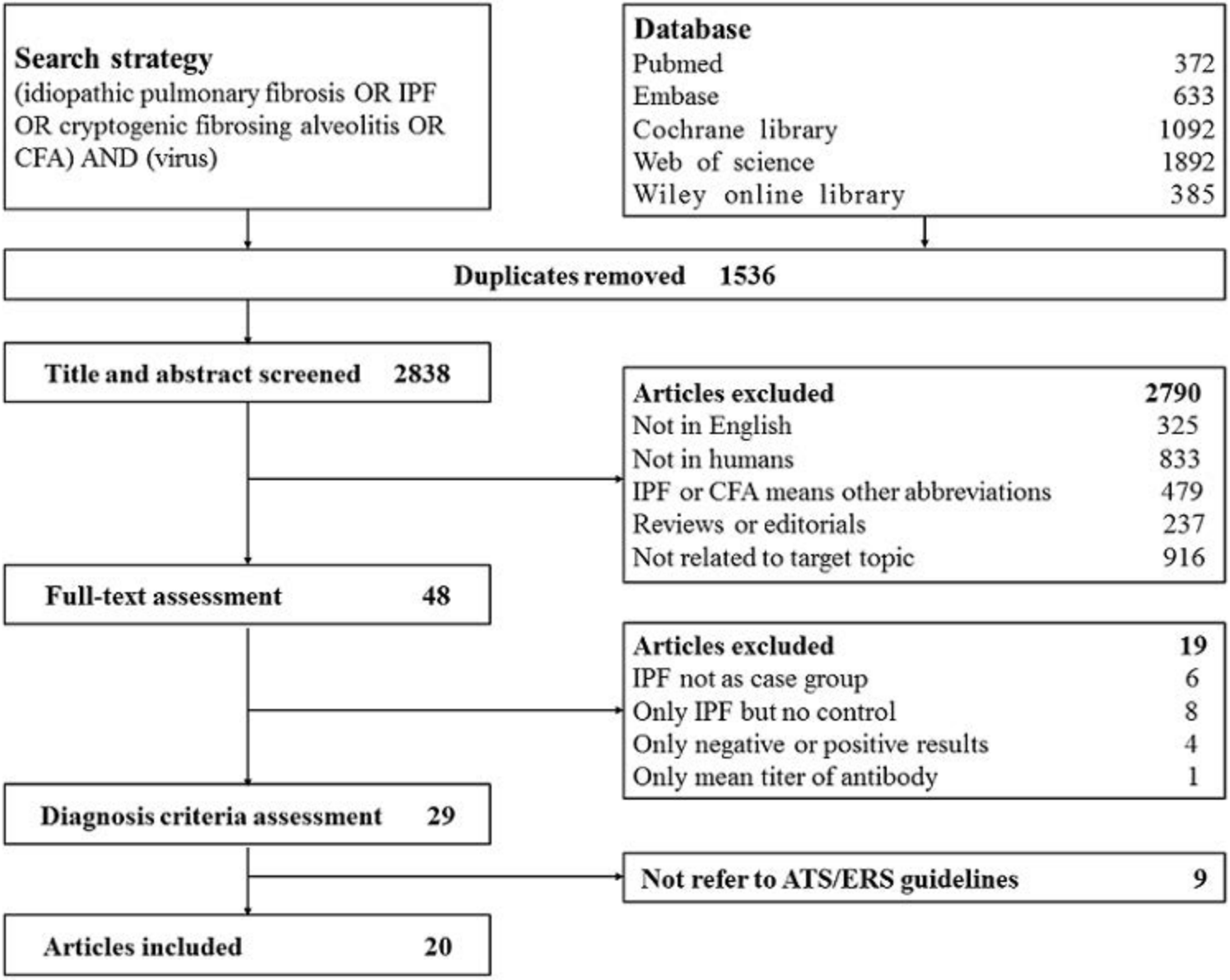
38. Naik PK, Moore BB. Viral infection and aging as cofactors for the development of pulmonary fibrosis. *Expert review of respiratory medicine.* 2010;4(6):759-771.
39. Roels E, Dourcy M, Holopainen S, et al. No Evidence of Herpesvirus Infection in West Highland White Terriers With Canine Idiopathic Pulmonary Fibrosis. *Vet Pathol.* 2016;53(6):1210-1212.
40. Virgin HW, Wherry EJ, Ahmed R. Redefining chronic viral infection. *Cell.* 2009;138(1):30-50.
41. Bowdish DME. The Aging Lung: Is Lung Health Good Health for Older Adults? *Chest.* 2019;155(2):391-400.
42. Qiao J, Zhang M, Bi J, et al. Pulmonary fibrosis induced by H5N1 viral infection in mice. *Respir Res.* 2009;10:107.
43. Mora AL, Torres-Gonzalez E, Rojas M, et al. Activation of alveolar macrophages via the alternative pathway in herpesvirus-induced lung fibrosis. *American journal of respiratory cell and molecular biology.* 2006;35(4):466-473.
44. Malizia AP, Keating DT, Smith SM, Walls D, Doran PP, Egan JJ. Alveolar epithelial cell injury with Epstein-Barr virus upregulates TGFbeta1 expression. *American journal of physiology. Lung cellular and molecular physiology.* 2008;295(3):L451-460.
45. Malizia AP, Lacey N, Walls D, Egan JJ, Doran PP. CUX1/Wnt signaling regulates epithelial mesenchymal transition in EBV infected epithelial cells. *Experimental cell research.* 2009;315(11):1819-1831.
46. Sides MD, Klingsberg RC, Shan B, et al. The Epstein-Barr virus latent membrane protein 1 and transforming growth factor--beta1 synergistically induce epithelial--mesenchymal transition in lung epithelial cells. *American journal of respiratory cell and molecular biology.* 2011;44(6):852-862.
47. Egan JJ, Adamali HI, Lok SS, Stewart JP, Woodcock AA. Ganciclovir antiviral therapy in advanced idiopathic pulmonary fibrosis: an open pilot study. *Pulmonary medicine.* 2011;2011:240805.
48. Mora AL, Torres-Gonzalez E, Rojas M, et al. Control of virus reactivation arrests pulmonary herpesvirus-induced fibrosis in IFN-gamma receptor-deficient mice. *Am J Respir Crit Care Med.* 2007;175(11):1139-1150.

Figure Legends

Figure 1. Search flow diagram for included studies in the meta-analysis.

Figure 2. Forest plot shows the pooled effect of OR for viral infection in idiopathic pulmonary fibrosis (IPF) patients and non-IPF controls. **a.** Viral infection was associated with a significant increase in the risk of IPF (OR: 3.48, 95%CI: 1.61-7.52, p=0.001). **b.** EBV infection was associated with significantly higher risk of IPF (OR: 9.83, 95%CI: 4.22-22.91, p<0.001). **c.** CMV infection significantly increased the risk of IPF (OR: 2.45, 95%CI: 1.29-4.66, p=0.006). **d.** No significant difference was found in HHV6 infection between IPF patients and controls (OR: 3.16, 95%CI: 0.41-24.50, p=0.270). **e.** HHV7 infection showed significantly higher risk of IPF (OR: 2.48, 95%CI: 1.14-5.40, p=0.022). **f.** HHV8 infection increased the risk of IPF (OR 8.88, 95%CI: 2.70-29.14, p<0.001).

Figure 3. Forest plot shows the pooled effect of OR for viral infection in idiopathic pulmonary fibrosis (IPF) patients with acute exacerbation and with multiple viral infections compared to non-IPF controls. **a.** The pooled effect showed that viral infection was not associated with acute exacerbation of IPF (OR: 0.99, 95%CI: 0.46-2.12, p=0.988). **b.** No significant increase was found in multiple viral infections between IPF patients and controls (OR: 3.16, 95%CI: 0.41-24.50, p=0.270).


Search strategy
(idiopathic pulmonary fibrosis OR IPF OR cryptogenic fibrosing alveolitis OR CFA) AND (virus)
Database
Pubmed 372
Embase 633
Cochrane library 1092
Web of science 1892
Wiley online library 385
Duplicates removed 1536
Title and abstract screened 2838
Articles excluded 2790
Not in English 325
Not in humans 833
IPF or CFA means other abbreviations 479
Reviews or editorials 237
Not related to target topic 916
Full-text assessment 48
Articles excluded 19
IPF not as case group 6
Only IPF but no control 8
Only negative or positive results 4
Only mean titer of antibody 1
Diagnosis criteria assessment 29
Not refer to ATS/ERS guidelines 9
Articles included 20

a

All Viruses

Study	OR(95% CI)	Weight %
Lasithiotaki et al. (2011)	0.38 (0.10, 1.41)	6.03
Lok et al. (2001)	51.00 (2.57, 1011.25)	3.49
Calabrese et al. (2013)	8.44 (2.32, 30.78)	6.08
Tang et al. (2003)	56.89 (6.62, 489.06)	4.66
Folcik et al. (2014)	1849.00 (35.06, 97503.81)	2.49
Tang et al. (2001)	82.50 (8.38, 812.63)	4.45
Tsukamoto et al. (2000)	6.40 (0.65, 62.84)	4.46
Stewart et al. (1999)	5.57 (1.52, 20.45)	6.07
Bando et al. (2008)	0.80 (0.14, 4.48)	5.36
Pozharskay et al. (2009)	19.00 (0.83, 434.45)	3.32
Wootton et al. (2011)	0.53 (0.19, 1.51)	6.46
Pulkkinen et al. (2012)	41.67 (1.96, 887.56)	3.40
Lawson et al. (2008)	38.29 (1.99, 737.37)	3.52
Kelly et al. (2002)	1.26 (0.35, 4.56)	6.10
Miyake et al. (2005)	1.01 (0.28, 3.60)	6.11
Dwomiczak et al. (2004)	1.36 (0.29, 6.42)	5.66
Rabea et al. (2015)	1.08 (0.41, 2.84)	6.58
Santos et al. (2013)	0.73 (0.15, 3.47)	5.64
Ushiki et al. (2014)	0.42 (0.03, 6.06)	3.89
Saraya et al. (2018)	0.93 (0.28, 3.07)	6.24
Overall (I-squared = 76.0%, p = 0.000)	3.48 (1.61, 7.52)	100.00

NOTE: Weights are from random effects analysis

1.0e-05 | 1 | 97504

b

EBV

Study	OR(95% CI)	Weight %
Lok et al. (2001)	51.00 (2.57, 1011.25)	6.16
Calabrese et al. (2013)	26.36 (1.52, 458.02)	6.60
Tang et al. (2003)	12.83 (3.17, 52.01)	15.36
Tang et al. (2001)	15.94 (2.92, 87.06)	12.82
Tsukamoto et al. (2000)	6.40 (0.65, 62.84)	9.04
Stewart et al. (1999)	5.57 (1.52, 20.45)	16.30
Pozharskaya et al. (2009)	19.00 (0.83, 434.45)	5.73
Pulkkinen et al. (2012)	176.33 (6.48, 4796.79)	5.26
Lawson et al. (2008)	11.52 (0.60, 221.75)	6.26
Kelly et al. (2002)	1.26 (0.35, 4.56)	16.46
Overall (I-squared = 41.5%, p = 0.081)	9.83 (4.22, 22.91)	100.00

NOTE: Weights are from random effects analysis

.00021 | 1 | 4797

c

CMV

Study	OR(95% CI)	Weight %
Calabrese et al. (2013)	1.53 (0.27, 8.78)	16.63
Tang et al. (2003)	14.43 (0.78, 265.99)	3.46
Tang et al. (2001)	17.73 (0.94, 334.26)	2.94
Pulkkinen et al. (2012)	5.48 (0.23, 127.73)	3.29
Lawson et al. (2008)	11.52 (0.60, 221.75)	3.47
Dworniczak et al. (2004)	1.36 (0.29, 6.42)	21.53
Santos et al. (2013)	0.42 (0.04, 4.18)	20.31
Ushiki et al. (2014)	0.15 (0.01, 3.71)	18.35
Saraya et al. (2018)	1.96 (0.26, 14.75)	10.04
Overall (I-squared = 24.1%, p = 0.229)	2.45 (1.29, 4.66)	100.00

.00299 | 1 | 334

d

HHV6

Study	OR(95% CI)	Weight %
Lasithiotaki et al. (2011)	0.54 (0.11, 2.55)	36.80
Calabrese et al. (2013)	5.83 (0.69, 49.43)	31.00
Pulkkinen et al. (2012)	13.33 (1.78, 100.14)	32.20
Overall (I-squared = 71.4%, p = 0.030)	3.16 (0.41, 24.50)	100.00

NOTE: Weights are from random effects analysis

.00999 | 1 | 100

e

HHV7

Study	OR(95% CI)	Weight %
Tang et al. (2003)	2.33 (0.74, 7.36)	47.44
Tang et al. (2001)	5.46 (1.01, 29.54)	16.09
Pulkkinen et al. (2012)	0.90 (0.10, 7.78)	20.99
Saraya et al. (2018)	1.96 (0.26, 14.75)	15.47
Overall (I-squared = 0.0%, p = 0.625)	2.48 (1.14, 5.40)	100.00

.0339 | 1 | 29.5

f

HHV8

Study	OR(95% CI)	Weight %
Tang et al. (2003)	9.58 (1.94, 47.43)	50.16
Tang et al. (2001)	13.85 (1.57, 121.99)	25.01
Lawson et al. (2008)	2.44 (0.11, 55.56)	24.82
Overall (I-squared = 0.0%, p = 0.662)	8.88 (2.70, 29.14)	100.00

.0082 | 1 | 122

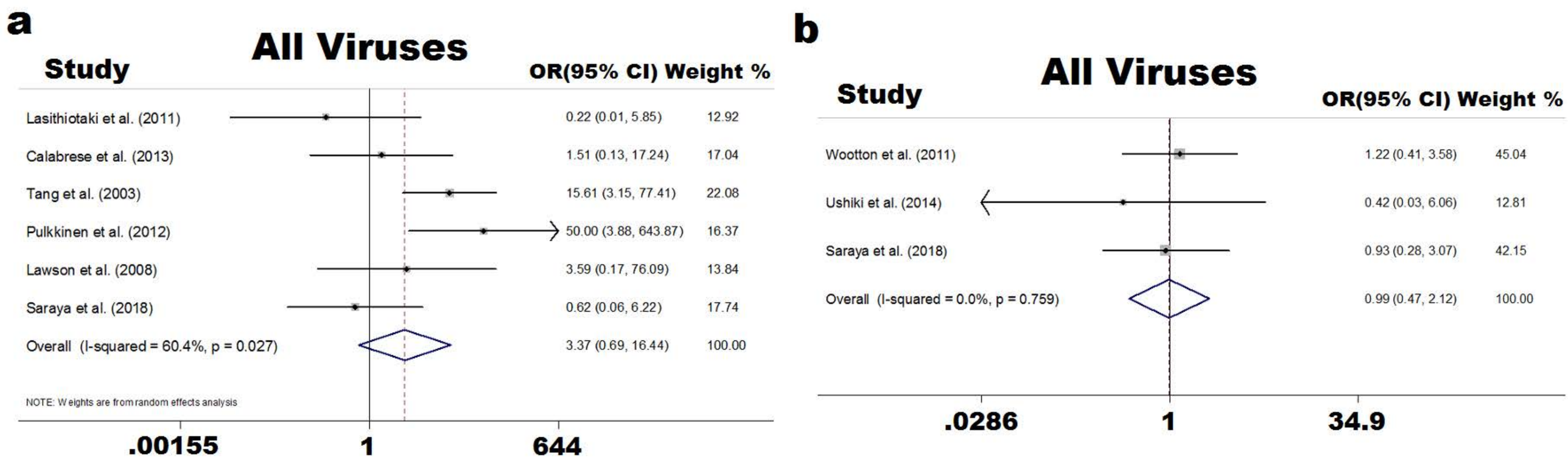

a
All Viruses
Study
OR(95% CI) Weight %
Lasithiotaki et al. (2011)
0.22 (0.01, 5.85)
12.92
Calabrese et al. (2013)
1.51 (0.13, 17.24)
17.04
Tang et al. (2003)
15.61 (3.15, 77.41)
22.08
Pulkkinen et al. (2012)
50.00 (3.88, 643.87)
16.37
Lawson et al. (2008)
3.59 (0.17, 76.09)
13.84
Saraya et al. (2018)
0.62 (0.06, 6.22)
17.74
Overall (I-squared = 60.4%, p = 0.027)
3.37 (0.69, 16.44)
100.00
NOTE: Weights are from random effects analysis
.00155
1
644
b
All Viruses
Study
OR(95% CI) Weight %
Wootton et al. (2011)
1.22 (0.41, 3.58)
45.04
Ushiki et al. (2014)
0.42 (0.03, 6.06)
12.81
Saraya et al. (2018)
0.93 (0.28, 3.07)
42.15
Overall (I-squared = 0.0%, p = 0.759)
0.99 (0.47, 2.12)
100.00
.0286
1
34.9

Table 1. Characteristics of included studies.

Study	Study design	Assessment NOS	Diagnosis	Country	Year
Lasithiotaki et al. 2011	Case-control	7	ATS/ERS statement, 2000	Greece	NA
Lok et al. 2001	Case-control	7	UIP pattern on histopathology and HRCT	UK	NA
Calabrese et al. 2013	Case-control	8	ATS/ERS statement, 2002	Italy	1998-2010
Tang et al. 2003	Case-control	8	UIP pattern on histopathology	USA	NA
Folcik et al. 2014	Case-control	7	ATS/ERS/JRS/ALAT statement, 2011	USA	NA
Tang et al. 2001	Case-control	6	UIP pattern on histopathology	USA	NA
Tsukamoto et al. 2000	Case-control	7	UIP pattern on histopathology	Japan	NA
Stewart et al. 1999	Case-control	8	UIP pattern on histopathology and HRCT	UK	NA
Bando et al. 2008	Case-control	6	ATS/ERS statement, 2000	Japan	1991-2003
Pozharskaya et al. 2009	Case-control	7	UIP pattern on histopathology	USA	NA

Wootton et al. 2011	Case-control	6	ATS/ERS statement, 2000 Collard et al. 2007	Korea; Japan	NA
Pulkkinen et al. 2012	Case-control	7	ATS/ERS statement, 2000/2002	Finland	NA
Lawson et al. 2008	Case-control	7	ATS/ERS statement, 2000/2002	USA	NA
Kelly et al. 2002	Case-control	7	UIP pattern on histopathology and HRCT	UK	NA
Miyake et al. 2005	Case-control	7	ATS/ERS statement, 2002	Japan	2001
Dworniczak et al. 2004	Case-control	6	ATS/ERS statement, 2000	Poland	NA
Rabea et al. 2015	Case-control	7	ATS/ERS statement, 2002	Egypt	NA
Santos et al. 2013	Case-control	7	ATS/ERS statement, 2002	Brazil	1993-2011
Ushiki et al. 2014	Case-control	7	ATS/ERS statement, 2002 Collard et al. 2007	Japan	2007-2011
Saraya et al. 2018	Case-control	8	Collard et al. 2007/2016	Japan	2012-2015

NOS, Newcastle-Ottawa Scale; ATS, American Thoracic Society; ERS, European Respiratory Society; JRS, Japanese Respiratory Society; ALAT, Latin American Thoracic Society; UIP, usual interstitial pneumonitis; UK, United Kingdom; USA, the United States of America; NA, not available; HRCT, high resolution computed tomography

Table 2. Characteristics of included participants.

Study	Virus	Sample	Method	Patients positive/total	Controls positive/total	Age, y	Gender, M/F	Smoking/ total	Immuno-suppression/ total
Lasithiotaki et al. 2011	HSV-1	lung	PCR	1/11	0/4	NA	NA	NA	NA
	HHV-6	BALF	PCR	5/13	8/13	64.95	18/8	14/26	NA
	CMV/HHV7/8	lung/BALF	PCR	0/24	0/17				
Lok et al. 2001	EBV	lung	PCR and IHC	8/14	0/19	55.55	19/14	NA	NA
Calabrese et al. 2013	EBV	lung	PCR and IHC	13/55	0/41	52	47/30	53/77	NA
	HHV-6			7/55	1/41				
	CMV			4/55	2/41				
	PVB19			0/55	1/41				
Tang et al. 2003	EBV	lung	PCR	21/33	3/25	52.41	32/18	NA	30/45
	CMV			7/33	0/25				
	HHV-7			14/33	6/25				
	HHV-8			15/33	2/25				
Folcik et al. 2014	HVS	lung	ISH and IHC	21/21	0/21	61.3	29/13	NA	NA
Tang et al. 2001	CMV	lung	PCR	7/23	0/19	NA	NA	NA	NA
	EBV			15/23	2/19				
	HHV-7			9/23	2/19				
	HHV-8			10/23	1/19				
	HSV-1/2 VZV/HHV-6			0/23	0/19				

Tsukamoto et al. 2000	EBV	lung	PCR	24/25	15/19	57.33	25/9	21/29	0/29
Stewart et al. 1999	EBV	lung	PCR	13/27	4/28	55.47	37/18	37/55	16/27
Bando et al. 2008	TTV	serum	PCR	55/57	138/142	65.29	147/52	153/199	0/199
Pozharskaya et al. 2009	EBV	lung	PCR	9/13	0/4	NA	NA	NA	NA
Wootton et al. 2011	TTV	BAL	PCR	12/83	7/29	64.06	87/25	80/112	NA
Pulkkinen et al. 2012	EBV HHV-6B HHV-7 CMV HSV-1 HSV-2	lung	PCR	11/12 10/12 2/12 2/12 1/12 1/12	0/11 3/11 2/11 0/11 0/11 0/11	58.71	18/3	11/21	2/21
Lawson et al. 2008	EBV CMV KSHV	lung	IHC	8/23 8/23 2/23	0/10 0/10 0/10	NA	NA	NA	NA
Kelly et al. 2002	EBV	serum	PCR	23/27	41/50	52.25	15/11	NA	36/77
Miyake et al. 2005	HCV	medical history	NA	7/104	4/60	NA	149/15	129/164	NA
Dworniczak et al. 2004	CMV	serum; blood leukocytes; BAL cells	PCR	12/16	11/16	38.85	21/11	14/32	0/32

Rabea et al. 2015	HCV	serum	ELISA	9/30	17/60	50.55	38/52	19/90	NA
Santos et al. 2013	MeV	lung	IHC	2/13	3/24	61.16	18/19	NA	NA
	CMV			1/13	4/24				
Ushiki et al. 2014	RSV-B	BAL	PCR	1/7	0/7	69.5	11/3	NA	1/14
	CMV			0/7	2/7				
	HHV-7			3/27	5/51				
	CMV			2/27	2/51				
Saraya et al. 2018	Influenza AH3	sputum; BALF; nasal swab	PCR	0/27	3/51	74.65	46/32	42/78	73/78
	Influenza H1N1			0/27	1/51				
	HPIV			1/27	1/51				
	HMPV			0/27	1/51				

y, year; M/F, male/female; NA, not available; PCR, polymerase chain reaction; BALF, bronchoalveolar lavage fluid; IHC, immunohistochemistry; ISH, in situ hybridization; BAL, bronchoalveolar lavage; ELISA, enzyme linked immunosorbent assay; HSV, herpes simplex virus; HHV, human herpesvirus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; PV, parvovirus; HVS, herpesvirus saimiri; VZV, varicella zoster virus; TTV, torque teno virus; KSHV, Kaposi's sarcoma herpesvirus; HCV, hepatitis C virus; MeV, measles virus; RSV, respiratory syncytial virus; AH3, influenza virus type A Hemagglutinin 3; H1N1, Hemagglutinin 1 Neuraminidase 1; HPIV, human parainfluenza virus; HMPV, human metapneumovirus

Supplementary Table 1. Newcastle-Ottawa Scale of quality assessment for included studies.

Study	Selection				Comparability[#]	Exposure			Star
	Is the case definition adequate?	Representativeness of the cases	Selection of Controls	Definition of Controls	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of exposure	Same method of ascertainment for cases and controls	Non-Response rate	
Lasithiotaki et al.[1] 2011	*	*	-	*	-	*	*	*	6
LOK et al.[2] 2001	*	*	-	*	*	*	*	*	7
Calabrese et al.[3] 2013	*	*	-	*	*	*	*	*	7
Tang et al.[4] 2003	*	*	-	*	*	*	*	*	7
Folcik et al.[5] 2014	*	*	-	*	**	*	*	*	8
Tang et al.[6] 2001	*	*		*	-	*	*	*	6
Tsukamoto et al.[7] 2000	*	*	-	*	*	*	*	*	7
Stewart et al.[8] 1999	*	*	-	*	**	*	*	*	8
Bando et al.[9] 2008	*	*	-	*	**	*	*	*	8

Pozharskaya et al.[10] 2009	*	*	-	*	-	*	*	*	6
Wootton et al.[11] 2011	*	*	-	*	**	*	*	*	8
Pulkkinen et al.[12] 2012	*	*	-	*	*	*	*	*	7
Lawson et al.[13] 2008	*	*	-	*	-	*	*	*	6
Kelly et al.[14] 2002	*	*	-	*	*	*	*	*	7
Miyake et al.[15] 2005	*	*	-	*	*	*	*	*	7
Dworniczak et al.[16] 2004	*	*	-	*	*	*	*	*	7
Rabea et al.[17] 2015	*	*	-	*	**	*	*	*	8
Santos et al.[18] 2013	*	*	-	*	**	*	*	*	8
Ushiki et al.[19] 2014	*	*	-	*	*	*	*	*	7
Saraya et al.[20] 2018	*	*	-	*	**	*	*	*	8

One star will be given if the age gap is less than or equal to 10 years between the case and control groups, and another star means the sex ratio (male/female) in case and control groups within 1/2-2; IPF, idiopathic pulmonary fibrosis

Supplementary Table 2. Classification of viruses from included studies*.

Virus	N	Abbreviation	Order	(Sub)family	Genus	Species
Epstein-Barr virus	10	EBV	Herpesvirales	Gammaherpesvirinae	Lymphocryptovirus	Human gammaherpesvirus 4
Cytomegalovirus	10	CMV	Herpesvirales	Betaherpesvirinae	Cytomegalovirus	Human betaherpesvirus 5
Human herpesvirus 7	5	HHV7	Herpesvirales	Betaherpesvirinae	Roseolovirus	Human betaherpesvirus 7
Human herpesvirus 6	4	HHV6	Herpesvirales	Betaherpesvirinae	Roseolovirus	Human herpesvirus 6
Human herpesvirus 8	3	HHV8	Herpesvirales	Gammaherpesvirinae	Rhadinovirus	Human gammaherpesvirus 8
Kaposi's sarcoma herpes virus	1	KSHV	Herpesvirales	Gammaherpesvirinae	Rhadinovirus	Human gammaherpesvirus 8
Herpes simplex virus type 1	3	HSV1	Herpesvirales	Alphaherpesvirinae	Simplexvirus	Human alphaherpesvirus 1
Herpes simplex virus type 2	2	HSV2	Herpesvirales	Alphaherpesvirinae	Simplexvirus	Human herpesvirus 2
Hepatitis C virus	2	HCV	Unassigned	Flaviviridae	Hepacivirus	Hepacivirus C
Torque teno virus	2	TTV	Unassigned	Anelloviridae	Alphatorquevirus	Torque teno virus
Herpesvirus saimiri	1	HVS	Herpesvirales	Gammaherpesvirinae	Rhadinovirus	Saimiriine herpesvirus 2
Measles virus	1	MeV	Mononegavirales	Paramyxoviridae	Morbillivirus	Measles morbillivirus
Respiratory syncytial virus	1	RSV	Mononegavirales	Pneumoviridae	Orthopneumovirus	Human orthopneumovirus
Human parvovirus B19	1	PVB19	Parvoviridae	Parvovirinae	Erythroparvovirus	Primate erythroparvovirus 1
Varicella zoster virus	1	VZV	Herpesvirales	Alphaherpesvirinae	Varicellovirus	Human alphaherpesvirus 3

Influenza virus AH3	1	-	Unassigned	Orthomyxoviridae	Alphainfluenzavirus	Influenza A virus
Influenza virus H1N1	1	-	Unassigned	Orthomyxoviridae	Alphainfluenzavirus	Influenza A virus
Human parainfluenza virus	1	HPIV	Mononegavirales	Paramyxoviridae	Respirovirus; or Rubulavirus	Human rubulavirus 1/2/3/4
Human metapneumovirus	1	HMPV	Mononegavirales	Pneumoviridae	Metapneumovirus	Human metapneumovirus

* Virus classification according to the report of International Committee on Taxonomy of Viruses (ICTV); N, the number of studies involved with this specific virus

Supplementary Table 3. Meta regression analyses of viral infection in IPF for age and gender.

	P value		
Virus	**Age**	**Gender**	**Age and Gender**
All viruses	0.328	0.959	0.484
EBV	0.312	0.239	0.726
CMV	0.741	0.663	0.936
HHV6	0.483	0.715	NA
HHV7	0.889	0.611	NA
HHV8	NA	NA	NA

IPF, idiopathic pulmonary fibrosis; EBV, Epstein-Barr virus; CMV, cytomegalovirus; HHV, human herpesvirus; NA, not available due to the insufficient number of studies

Supplementary Table 4. Subgroup analyses of OR for viral infection in participants with or without IPF.

	Studies/participants, n/N	OR [95% CI]	P value	P value for interaction
		All viruses		
Age, y				
<60	9/508	5.24[2.00-13.75]	0.001	0.168
≥60	7/523	0.99[0.31-3.13]	0.986	
Gender, M/N				
<0.65	7/469	3.09[1.00-9.52]	0.050	0.697
≥0.65	10/726	2.09[0.72-6.09]	0.177	
		EBV		
Age, y				
<55	3/231	6.13[0.90-41.84]	0.064	0.656
≥55	4/155	14.49[3.26-64.35]	<0.001	
Gender, M/N				
<0.65	4/264	9.06[1.55-52.86]	0.014	0.910
≥0.65	3/122	11.33[2.06-62.36]	0.005	
		CMV		
Age, y				
<60	4/209	2.73[1.06-7.06]	0.038	0.196
≥60	3/129	0.63[0.18-2.27]	0.483	
Gender, M/N				
<0.65	4/269	2.05[0.81-5.18]	0.129	0.521
≥0.65	3/69	1.16[0.37-3.62]	0.799	
		Multiple viral infections*		
Age, y				
<60	3/177	10.86[1.82-64.71]	0.009	0.516
≥60	2/119	0.44[0.07-2.90]	0.393	

Gender, M/N				
<0.65	3/232	2.05[0.02-758.61]	0.327	0.996
≥0.65	2/64	2.83[0.35-22.78]	0.631	

OR, odds ratio; IPF, idiopathic pulmonary fibrosis; y, years; M/N, men/total; EBV, Epstein-Barr virus; CMV, Cytomegalovirus; * two or more viruses simultaneously detected as positive in one participant

Supplementary Table 5. Comparison of results between studies involving "IPF" and those referring to ATS/ERS statements.

	OR [95% CI], P value	
Virus	**Studies involving "IPF"**	**Studies referring to ATS/ERS guidelines**
All viruses	3.01 [1.59-5.68], p=0.001	3.48 [1.61-7.52], p=0.001
EBV	4.93 [1.89-12.87], p=0.001	9.83 [4.22-22.91], p<0.001
CMV	2.45 [1.29-4.66], p=0.006	2.45 [1.29-4.66], p=0.006
HHV6	3.16 [0.41-24.50], p=0.270	3.16 [0.41-24.50], p=0.270
HHV7	2.48 [1.14-5.40], p=0.022	2.48 [1.14-5.40], p=0.022
HHV8	8.88 [2.70-29.14], p<0.001	8.88 [2.70-29.14], p<0.001

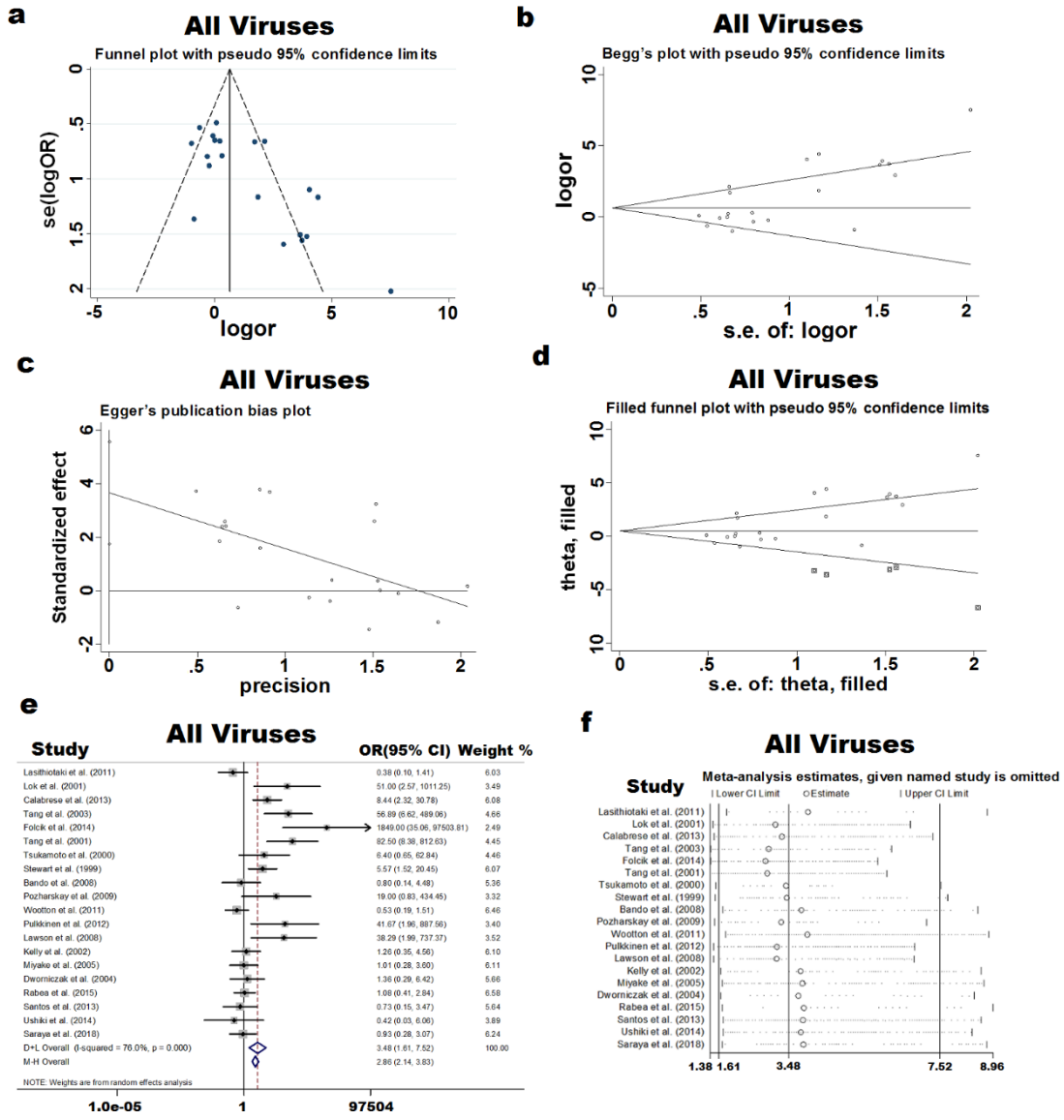
OR, odds ratio; IPF, idiopathic pulmonary fibrosis; ATS, American Thoracic Society; ERS, European Respiratory Society; EBV, Epstein-Barr virus; CMV, cytomegalovirus; HHV, human herpesvirus

Supplementary Table 6. Characteristics of studies and participants not referring to ATS/ERS statements.

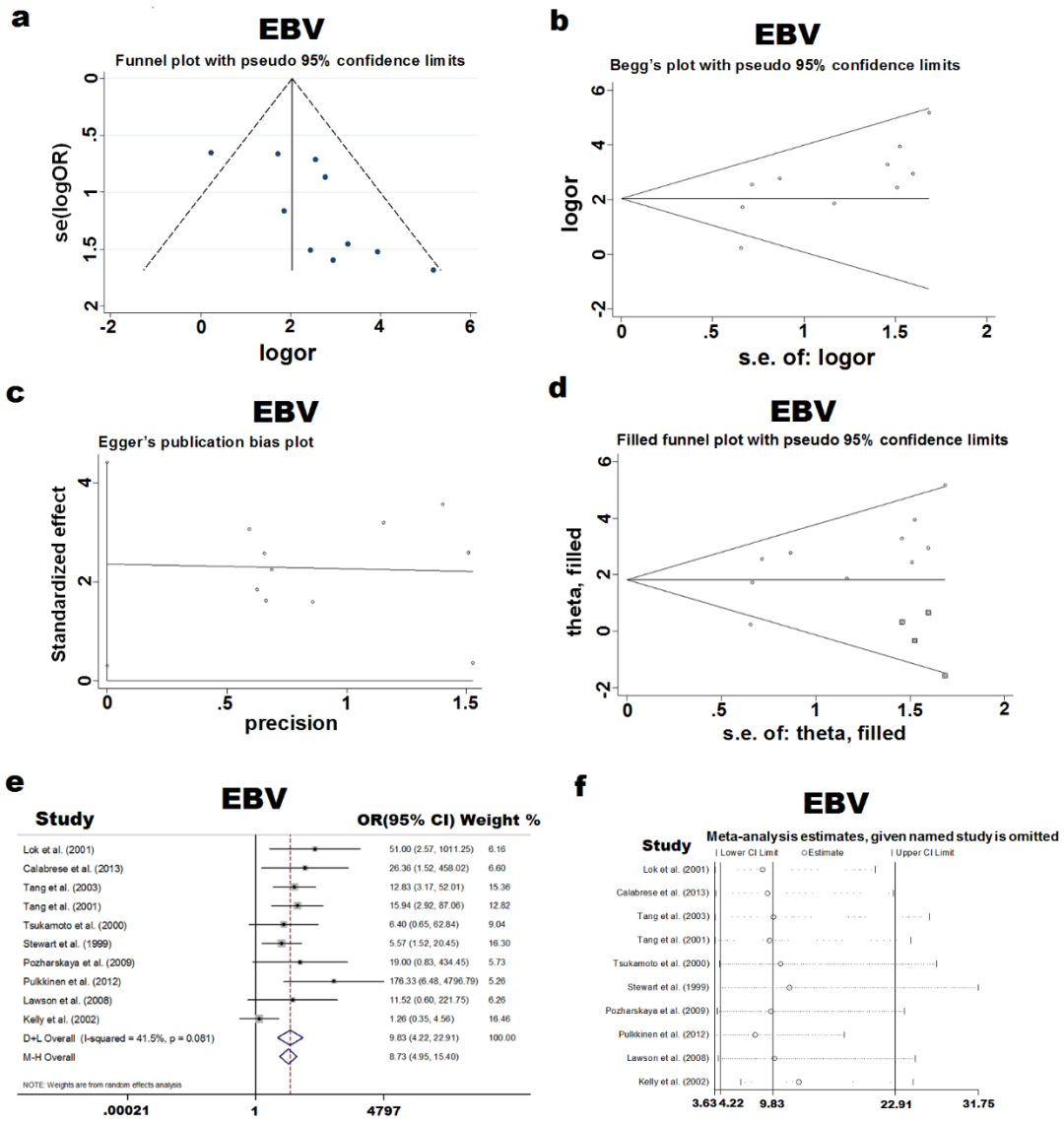
Study	Diagnosis	Assessment NOS	Virus	Sample	Method	Patients positive/total	Controls positive/total	Age, y	Gender, M/F
Wangoo et al.[21] 1997	Histology	6	EBV	lung	IHC, ISH, and PCR	0/12	1/13	NA	NA
Kuwano et al.[22] 1997	Medical history, physical examination, laboratory tests, chest radiography, pulmonary function tests, and pulmonary biopsies	7	ADV	lung	PCR and ISH	3/19	7/30	52.61	24/25
Egan et al.[23] 1995	Typical histological and clinical picture	8	EBV	lung	IHC	14/20	2/21	59.54	31/10
Ueda et al.[24] 1992	Symptoms of progressive dyspnea and cough; audible fine crackles; chest radiograph; pulmonary function tests; no manifestations compatible with other interstitial lung diseases	8	HCV	serum	ELISA and RIBA	19/66	346/9464	61.5	46/20
Meliconi et al.[25] 1996	Clinical, radiological, physiological, and histological grounds	8	HCV	serum	ELISA and RIBA	8/60	22/4744	62.34	106/84
Matsuyama et al.[26] 2003	Chest radiograph, physical examination, lung function, pathological findings and no known diseases	7	HTLV-1	serum	serologic test and western blot	18/72	772/4782	62.4	NA

Vergnon et al.[27] 1984	Clinical, chest X-ray, lung function, and histology	8	EBV	serum	serologic test (IgM against VCA)	3/13	2/12	62.4	11/14
Barbera et al.[28] 1992	Histopathology	8	EBV	lung	ISH	2/10	9/14	56.83	12/12
Kaan et al.[29] 1997	Histopathology	8	EBV	lung	IHC	3/9	13/22	59.44	NA

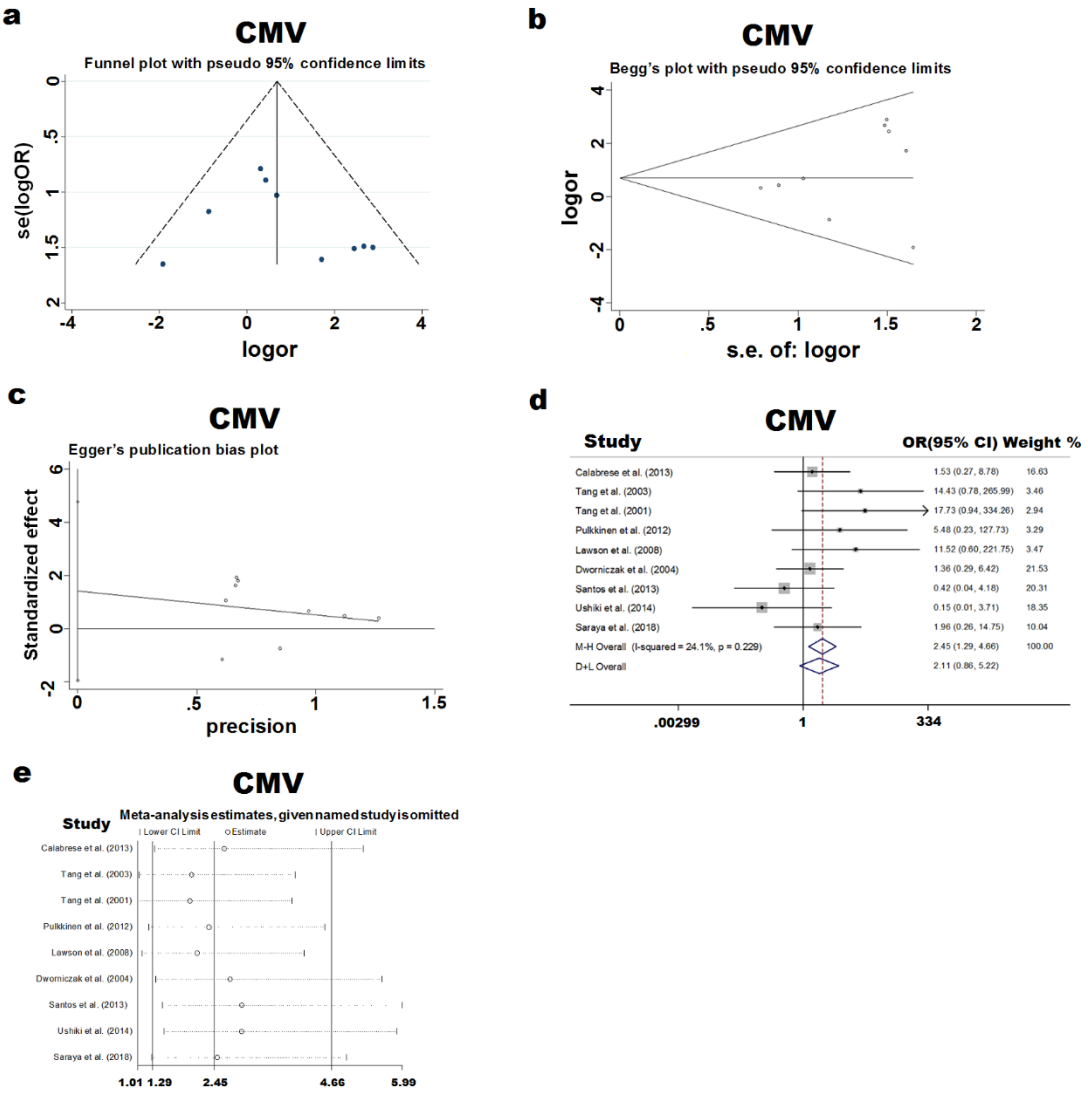
ATS, American Thoracic Society; ERS, European Respiratory Society; NOS, Newcastle-Ottawa Scale; y, year; M/F, male/female; NA, not available; IHC, immunohistochemistry; ISH, in situ hybridization; PCR, polymerase chain reaction; ELISA, enzyme linked immunosorbent assay; RIBA, recombinant immunoblotting assay; VCA, viral capsid antigen; EBV, Epstein-Barr virus; ADV, adenovirus; HCV, hepatitis C virus; HTLV, human T lymphotrophic virus



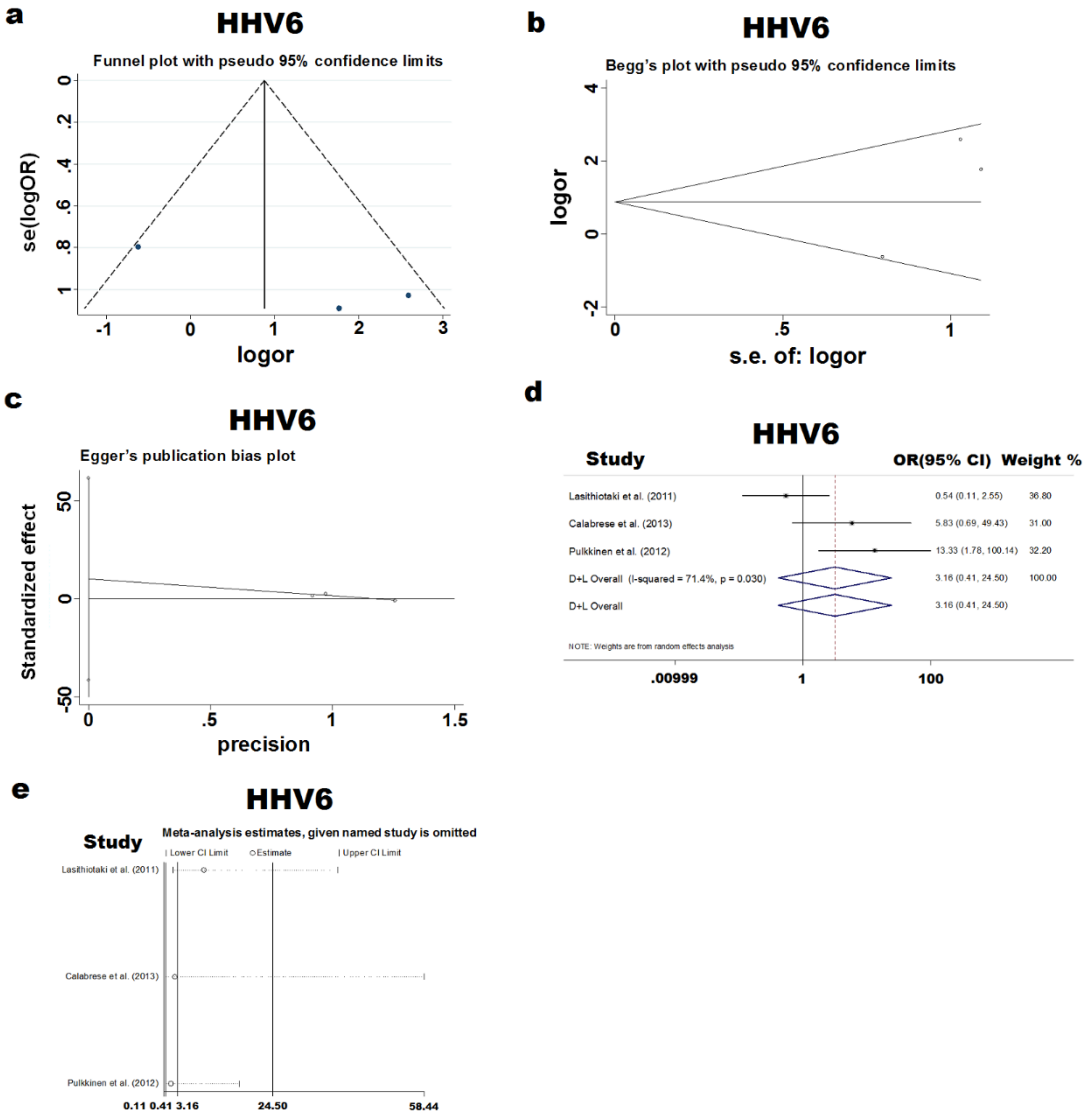
Supplementary Figure 1. a. Funnel plot showed a certain extent publication bias for all viruses. **b.** Begg's funnel plot showed a certain extent publication bias of OR for all viruses. **c.** Egger's funnel plot showed a certain extent publication bias of OR for all viruses. **d.** Begg's funnel plot using trim and fill method. Circle indicates included individual study and box the hypothetical study estimated by trim and fill method. **e.** The similarity between random and fixed effect models showed no significant influence on the pooled effect. **f.** Sensitive analysis of OR showed no significant difference.



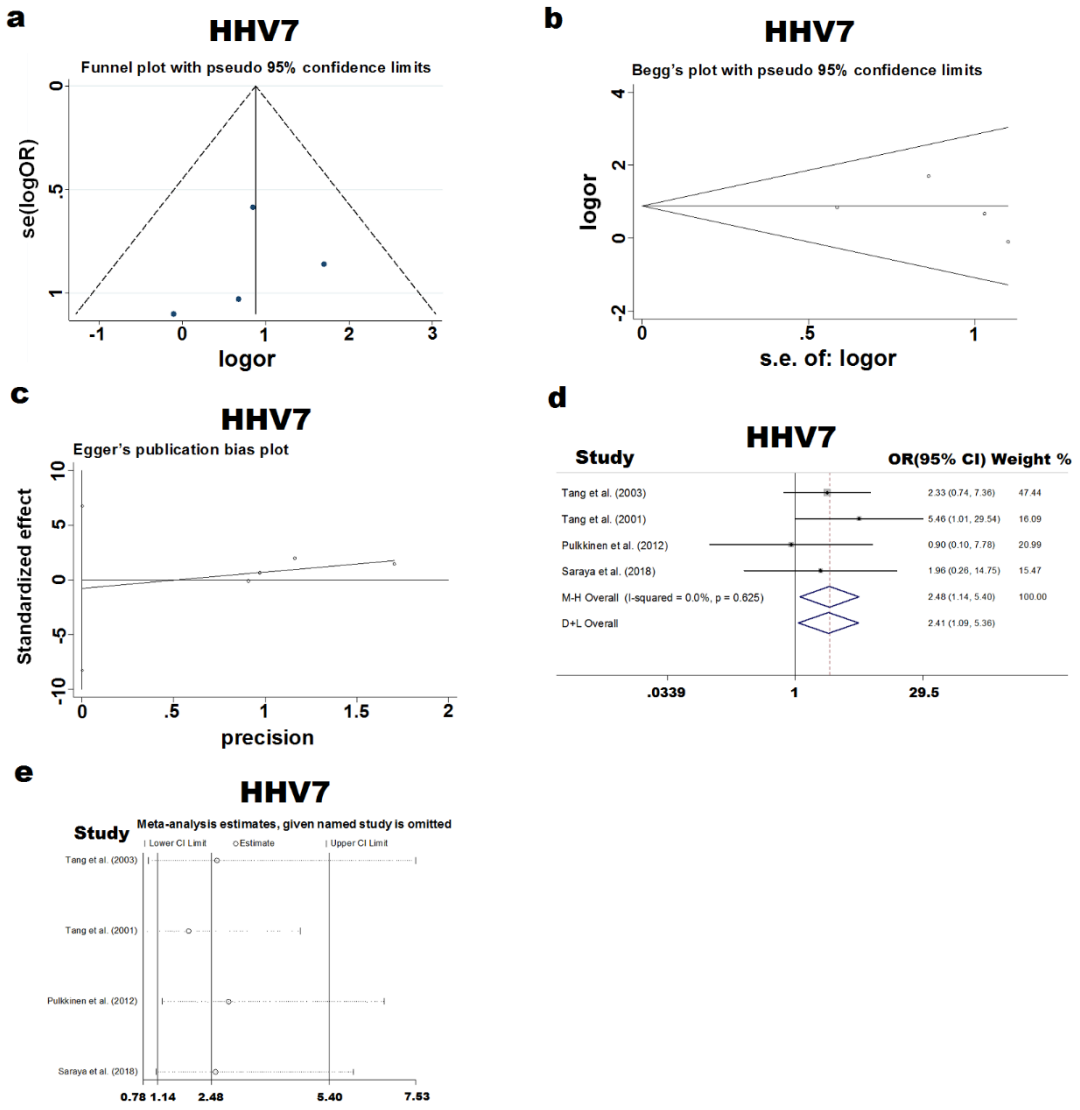
Supplementary Figure 2. a. Funnel plot showed a certain extent publication bias for EBV. **b.** Begg's funnel plot showed a certain extent publication bias of OR for EBV. **c.** Egger's funnel plot showed a certain extent publication bias of OR for EBV. **d.** Begg's funnel plot using trim and fill method. Circle indicates included individual study and box the hypothetical study estimated by trim and fill method. **e.** The similarity between random and fixed effect models showed no significant influence on the pooled effect. **f.** Sensitive analysis of OR showed no significant difference.



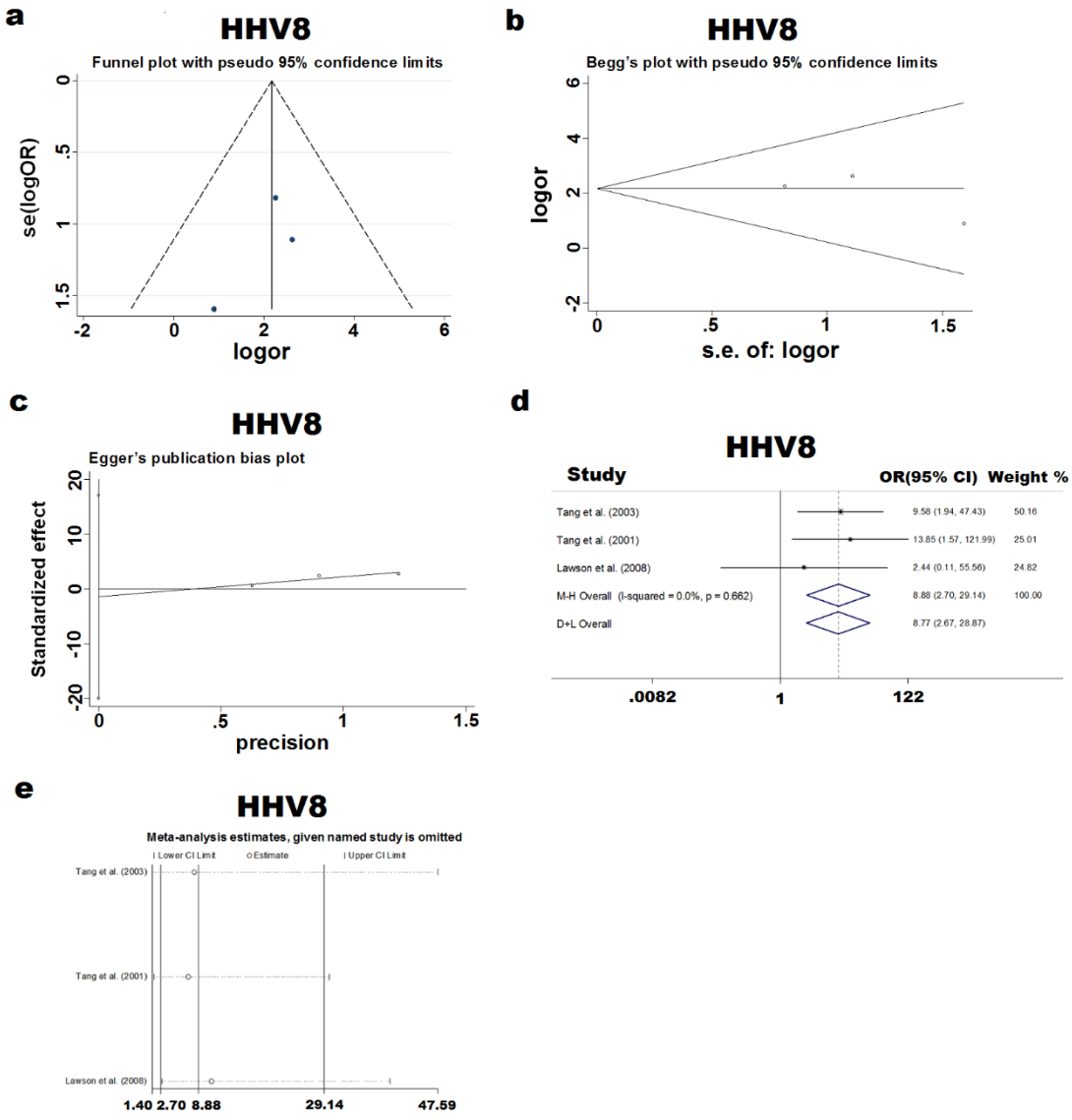
Supplementary Figure 3. a. Funnel plot showed no publication bias for CMV. **b.** Begg's funnel plot showed no publication bias of OR for CMV. **c.** Egger's funnel plot showed no publication bias of OR for CMV. **d.** The similarity between random and fixed effect models showed no significant influence on the pooled effect. **e.** Sensitive analysis of OR showed no significant difference.



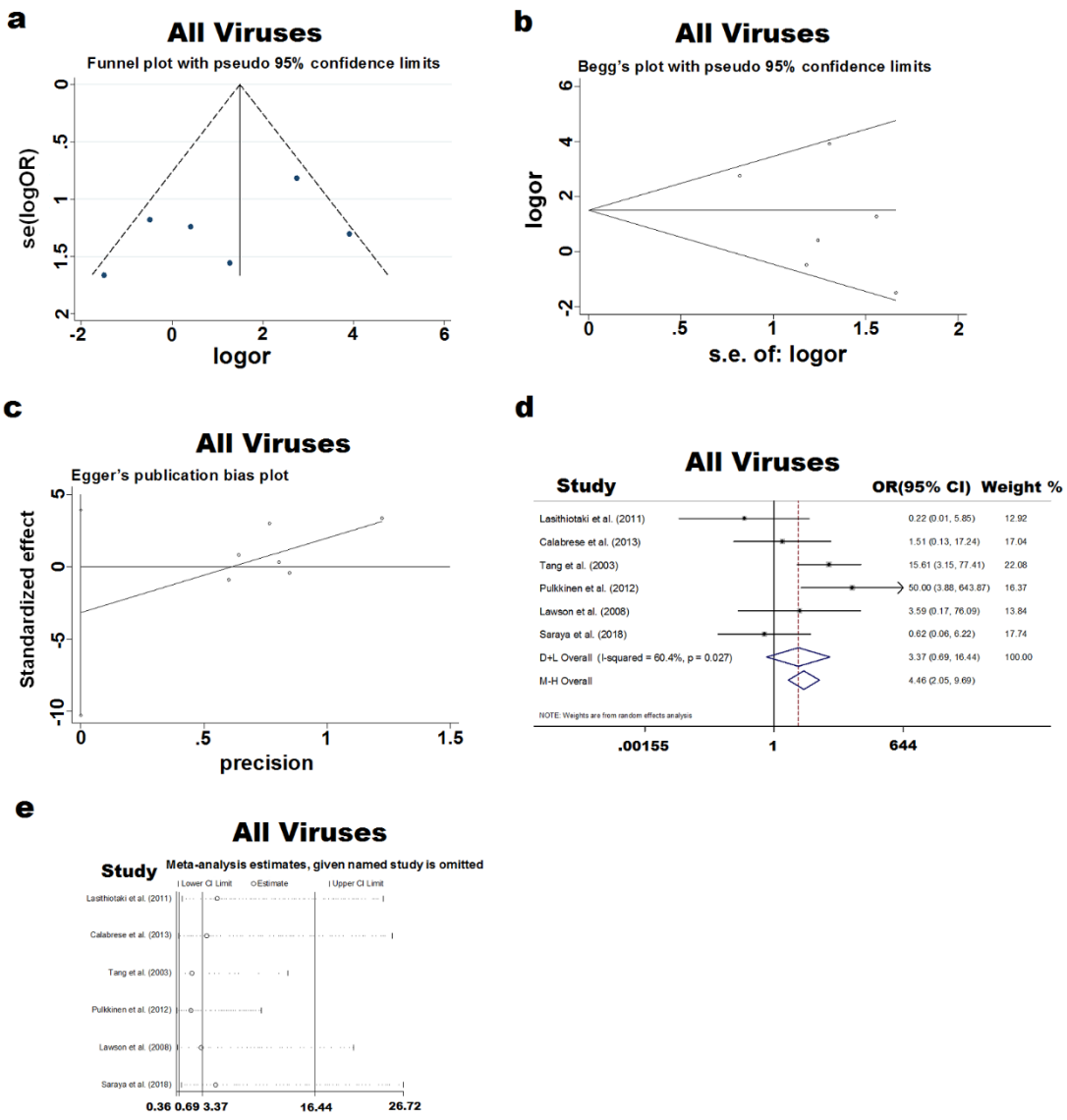
Supplementary Figure 4. a. Funnel plot showed no publication bias for HHV6. **b.** Begg's funnel plot showed no publication bias of OR for HHV6. **c.** Egger's funnel plot showed no publication bias of OR for HHV6. **d.** The similarity between random and fixed effect models showed no significant influence on the pooled effect. **e.** Sensitive analysis of OR showed no significant difference.



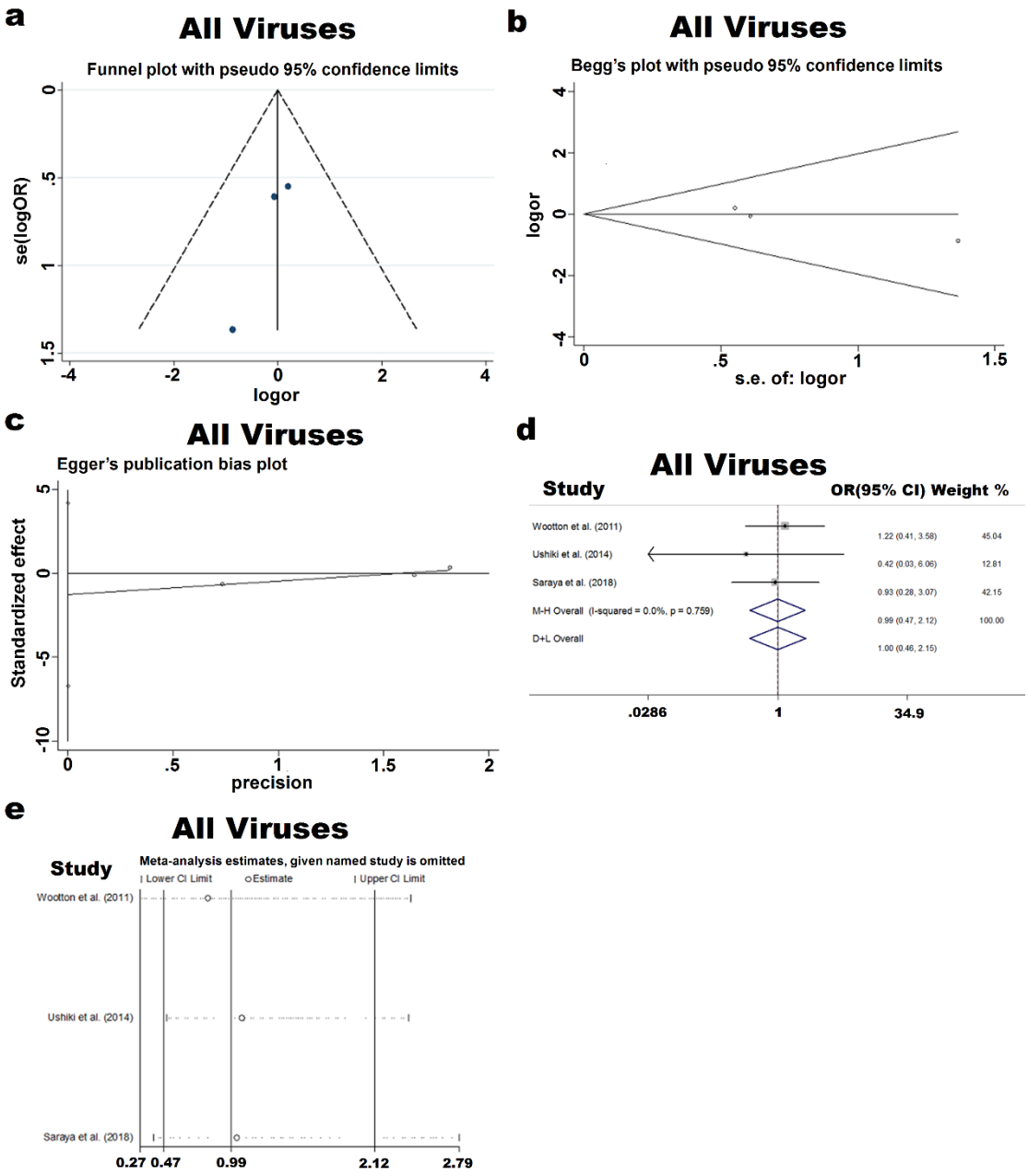
Supplementary Figure 5. a. Funnel plot showed no publication bias for HHV7. **b.** Begg's funnel plot showed no publication bias of OR for HHV7. **c.** Egger's funnel plot showed no publication bias of OR for HHV7. **d.** The similarity between random and fixed effect models showed no significant influence on the pooled effect. **e.** Sensitive analysis of OR showed no significant difference.



Supplementary Figure 6. a. Funnel plot showed no publication bias for HHV8. **b.** Begg's funnel plot showed no publication bias of OR for HHV8. **c.** Egger's funnel plot showed no publication bias of OR for HHV8. **d.** The similarity between random and fixed effect models showed no significant influence on the pooled effect. **e.** Sensitive analysis of OR showed no significant difference.



Supplementary Figure 7. a. Funnel plot showed no publication bias for multiple viral infections. **b.** Begg's funnel plot showed no publication bias of OR for multiple viral infections. **c.** Egger's funnel plot showed no publication bias of OR for multiple viral infections. **d.** The similarity between random and fixed effect models showed no significant influence on the pooled effect. **e.** Sensitive analysis of OR showed no significant difference.



Supplementary Figure 8. a. Funnel plot showed no publication bias in acute exacerbation of IPF for all viruses. **b.** Begg's funnel plot showed no publication bias of OR in acute exacerbation of IPF for all viruses. **c.** Egger's funnel plot showed no publication bias of OR in acute exacerbation of IPF for all viruses. **d.** The similarity between random and fixed effect models showed no significant influence on the pooled effect. **e.** Sensitive analysis of OR showed no significant difference.

Reference

1. Lasithiotaki I, Antoniou KM, Vlahava VM, et al. Detection of herpes simplex virus type-1 in patients with fibrotic lung diseases. *PLoS One.* 2011;6(12):e27800.
2. Lok SS, Stewart JP, Kelly BG, Hasleton PS, Egan JJ. Epstein-Barr virus and wild p53 in idiopathic pulmonary fibrosis. *Respir Med.* 2001;95(10):787-791.
3. Calabrese F, Kipar A, Lunardi F, et al. Herpes virus infection is associated with vascular remodeling and pulmonary hypertension in idiopathic pulmonary fibrosis. *PLoS One.* 2013;8(2):e55715.
4. Tang YW, Johnson JE, Browning PJ, et al. Herpesvirus DNA Is Consistently Detected in Lungs of Patients with Idiopathic Pulmonary Fibrosis. *Journal of Clinical Microbiology.* 2003;41(6):2633-2640.
5. Folcik VA, Garofalo M, Coleman J, et al. Idiopathic pulmonary fibrosis is strongly associated with productive infection by herpesvirus saimiri. *Mod Pathol.* 2014;27(6):851-862.
6. Tang YW, Johnson J, Cruz-Gervis R, et al. Increased detection of herpesvirus DNA in idiopathic pulmonary fibrosis. *Chest.* 2001;120(1 Suppl):74S-75S.
7. Tsukamoto K, Hayakawa H, Sato A, Chida K, Nakamura H, Miura K. Involvement of Epstein-Barr virus latent membrane protein 1 in disease progression in patients with idiopathic pulmonary fibrosis. *Thorax.* 2000;55(11):958-961.
8. Stewart JP, Egan JJ, Ross AJ, et al. The detection of Epstein-Barr virus DNA in lung tissue from patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 1999;159(4 Pt 1):1336-1341.
9. Bando M, Takahashi M, Ohno S, et al. Torque teno virus DNA titre elevated in idiopathic pulmonary fibrosis with primary lung cancer. *Respirology.* 2008;13(2):263-269.
10. Pozharskaya V, Torres-Gonzalez E, Rojas M, et al. Twist: a regulator of epithelial-mesenchymal transition in lung fibrosis. *PLoS One.* 2009;4(10):e7559.
11. Wootton SC, Kim DS, Kondoh Y, et al. Viral infection in acute exacerbation of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* 2011;183(12):1698-1702.
12. Pulkkinen V, Salmenkivi K, Kinnula VL, et al. A novel screening method detects herpesviral DNA in the idiopathic pulmonary fibrosis lung. *Ann Med.* 2012;44(2):178-186.
13. Lawson WE, Crossno PF, Polosukhin VV, et al. Endoplasmic reticulum stress in alveolar epithelial cells is prominent in IPF: association with altered surfactant protein processing and herpesvirus infection. *Am J Physiol Lung Cell Mol Physiol.* 2008;294(6):L1119-1126.
14. Kelly BG, Lok SS, Hasleton PS, Egan JJ, Stewart JP. A Rearranged Form of Epstein–Barr Virus DNA Is Associated with Idiopathic Pulmonary Fibrosis. *American Journal of Respiratory and Critical Care Medicine.* 2002;166(4):510-513.
15. Miyake Y, Sasaki S, Yokoyama T, et al. Case-control study of medical history and idiopathic pulmonary fibrosis in Japan. *Respirology.* 2005;10(4):504-509.
16. Dworniczak S, Ziora D, Kapral M, et al. Human cytomegalovirus DNA level in patients with idiopathic pulmonary fibrosis. *J Physiol Pharmacol.* 2004;55 Suppl 3:67-75.
17. Rabea AEM, Zidan M, Daabis R, El Sayed P, Samir S. Prevalence of chronic hepatitis C virus (HCV) infection in patients with idiopathic pulmonary fibrosis. *Egyptian Journal of Chest Diseases and Tuberculosis.* 2015;64(4):907-913.
18. Santos GC, Parra ER, Stegun FW, Cirqueira CS, Capelozzi VL. Immunohistochemical detection of virus through its nuclear cytopathic effect in idiopathic interstitial pneumonia

other than acute exacerbation. *Braz J Med Biol Res.* 2013;46(11):985-992.

19. Ushiki A, Yamazaki Y, Hama M, Yasuo M, Hanaoka M, Kubo K. Viral infections in patients with an acute exacerbation of idiopathic interstitial pneumonia. *Respir Investig.* 2014;52(1):65-70.
20. Saraya T, Kimura H, Kurai D, et al. Clinical significance of respiratory virus detection in patients with acute exacerbation of interstitial lung diseases. *Respir Med.* 2018;136:88-92.
21. Wangoo A, Shaw RJ, Diss TC, Farrell PJ, du Bois RM, Nicholson AG. Cryptogenic fibrosing alveolitis: lack of association with Epstein-Barr virus infection. *Thorax.* 1997;52(10):888-891.
22. Kuwano K, Nomoto Y, Kunitake R, et al. Detection of adenovirus E1A DNA in pulmonary fibrosis using nested polymerase chain reaction. *European Respiratory Journal.* 1997;10(7):1445-1449.
23. Egan JJ, Stewart JP, Hasleton PS, Arrand JR, Carroll KB, Woodcock AA. Epstein-Barr virus replication within pulmonary epithelial cells in cryptogenic fibrosing alveolitis. *Thorax.* 1995;50(12):1234-1239.
24. Ueda T, Ohta K, Suzuki N, et al. Idiopathic pulmonary fibrosis and high prevalence of serum antibodies to hepatitis C virus. *Am Rev Respir Dis.* 1992;146(1):266-268.
25. Meliconi R, Andreone P, Fasano L, et al. Incidence of hepatitis C virus infection in Italian patients with idiopathic pulmonary fibrosis. *Thorax.* 1996;51(3):315-317.
26. Matsuyama W, Kawabata M, Mizoguchi A, Iwami F, Wakimoto J, Osame M. Influence of human T lymphotrophic virus type I on cryptogenic fibrosing alveolitis - HTLV-I associated fibrosing alveolitis: proposal of a new clinical entity. *Clin Exp Immunol.* 2003;133(3):397-403.
27. Vergnon JM, Vincent M, de The G, Mornex JF, Weynants P, Brune J. Cryptogenic fibrosing alveolitis and Epstein-Barr virus: an association? *Lancet.* 1984;2(8406):768-771.
28. Barbera JA, Hayashi S, Hegele RG, Hogg JC. Detection of Epstein-Barr virus in lymphocytic interstitial pneumonia by in situ hybridization. *Am Rev Respir Dis.* 1992;145(4 Pt 1):940-946.
29. Kaan PM, Hegele RG, Hayashi S, Hogg JC. Expression of bcl-2 and Epstein-Barr virus LMP1 in lymphocytic interstitial pneumonia. *Thorax.* 1997;52(1):12-16.